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Shedding of foodborne pathogens and microbial carcass contamination of hunted wild ruminants

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Shedding of foodborne pathogens and microbial carcass contamination of hunted wild ruminants

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1. Summary

To assess the shedding of selected bacterial foodborne pathogens, fecal samples from 239 hunted wild red deer, roe deer, chamois, and ibex were examined. All samples tested negative for *Salmonella* spp. and *L. monocytogenes* but other *Listeria* species were occasionally found. Of the 239 fecal samples, 32.6% tested positive for *stx* (Shiga toxins), 6.7% for *eae* (intimin) and 13.8% for both *stx* and *eae* genes. Amongst the 56 isolated Shiga toxin-producing *Escherichia coli* (STEC) strains, 44.6% harbored genes for the Stx2 group, 30.4% for the Stx1 group, and 21.4% for both Stx1 and Stx2. Only two of these strains harbored *eae*. Hence, wild ruminants constitute a reservoir for STEC but further characterization data of the isolated strains are required to assess their actual human pathogenicity. In addition, 328 carcasses from hunted wild red deer, roe deer, and chamois were examined for total viable counts (TVC) and *Enterobacteriaceae* by swabbing. For the examined species, average total viable counts (4.0–4.2 log CFU cm^{-2}) and average *Enterobacteriaceae* counts/detection rates (2.3–2.6 log CFU cm^{-2}; 87.5–90%) were at comparable levels. On the other hand, the microbial status of carcasses differed between certain abattoirs by several orders of magnitude. Strict compliance with good hunting and hygiene practices during any step from shooting, through evisceration in the field, to dehiding, cooling, and processing is therefore of central importance to avoid contaminations and to prevent foodborne pathogens carried by the animals from entering the food chain.

Keywords: Hunted wild ruminants; Fecal shedding; *Salmonella*; *Listeria monocytogenes*; Shiga toxin-producing *Escherichia coli*; Carcass contamination

2. Introduction

Game meat is appreciated for its nutritional, physiological, and dietary properties. World game meat production is estimated to be over 1.8 million tons annually (FAO, 2011). In central Europe, annual per capita consumption is about one kilogram (Atanassova et al., 2008; Membré et al., 2011) and the demand for game meat has increased in recent years. Of the game consumed in Switzerland, 20–25% originates from domestic production, mainly domestic hunts (Proviande, 2010). With regard to wild ruminants hunted in the year 2010, 9'016 red deer, 39'664 roe deer, 13'339 chamois, and 1'074 ibex were shot in Switzerland (BAFU, 2010). In the European Union, wild game and game meat are covered by the food hygiene legislation (Reg. [EC] No 852/2004 and 853/2004). Hunters selling game to processing companies are considered food business operators with respective responsibilities in view of food safety or traceability (Atanassova et al., 2008).

The microbial conditions of carcasses and meat obtained from hunted animals can vary greatly. Several highly variable factors influence both the initial contamination and the potential subsequent growth of the bacterial flora (Gill, 2007; Paulsen, 2011). These factors include but are not limited to: the health of the animal, the types of microorganisms present, the circumstances in which the animal is killed, the evisceration in the field, the time before cooling, the practice of hanging unskinned carcasses, or the conditions under which carcasses are transported, stored, and processed. Thereby, it must be considered that healthy animals were recognized in recent years as important carriers of bacterial pathogens causing human illness (EFSA/ECDC, 2011). Such zoonotic pathogens from the intestine or the hide might enter the food chain by direct or indirect fecal contamination if good hunting and hygiene practices are not warranted. Because recent data from healthy wild ruminants are limited, the aim of this study was (i) to assess the shedding of selected bacterial foodborne pathogens in hunted wild red deer, roe deer, chamois, and ibex, and (ii) to evaluate the microbial contamination of carcasses from hunted wild red deer, roe deer, and chamois.

3. Materials and methods

3.1. Sampling

This study was based on investigations carried out during three months of the hunting season 2011 (September–November). Samples originated from wild red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), chamois (*Rupicapra rupicapra*), and ibex (*Capra ibex*) hunted in the central and eastern part of Switzerland. In total, 239 fecal samples (red deer, roe deer, chamois, and ibex) and 328 carcasses (red deer, roe deer, and chamois) were examined.

Fecal samples originated from 84 red deer, 64 roe deer, 64 chamois, and 27 ibex. State gamekeepers and hunters collected the samples in the field immediately after shooting and evisceration. From each sampled animal, sex, age, and location of hunting were recorded. After opening of the large intestine, fecal matter was collected from the colon, placed into sterile tubes, and stored frozen.

In addition, carcasses from 136 red deer, 122 roe deer, and 70 chamois were sampled. Carcasses originated from six small-scale abattoirs A–F (five located in the eastern part and one located in the central part of Switzerland). Eviscerated but unskinned game animals arrived within 48 h after shooting at these abattoirs where they were dehided, washed (in certain abattoirs), and stored in the chiller. Carcass samples were obtained from the neck, brisket, flank, and rump area (each of 40 cm^2) using the wet-dry double swab technique. Selected carcasses have thereby been in the chiller not longer than 72 h. Swabs from each carcass were pooled and transported chilled.

In the laboratory, fecal samples were analyzed qualitatively for *Salmonella*, *Listeria (L.) monocytogenes*, and *Escherichia (E.) coli* harboring *stx* (Shiga toxins) and *eae* (intimin). Pooled carcass samples were analyzed for total viable counts (TVC) and *Enterobacteriaceae*.

3.2. *Salmonella* spp.

Examination for *Salmonella* spp. was done in accordance with ISO 6579:09.2006. From each fecal sample, 1 g was enriched (24 h, 37 °C) in 10 ml of buffered peptone water (Oxoid AG, Pratteln, CH). Three drops of the first enrichment were incubated for 48 h at 41.5 °C onto modified semi-solid Rappaport Vassiliadis agar (Oxoid AG). Presumptive positive samples were subcultured (24 h, 37 °C) onto xylose-lysine-desoxycholate agar and mannitol lysine crystal violet brilliant green agar (Oxoid AG).

Suspicious colonies were tested for biochemical properties of *Salmonella*.

3.3. *Listeria monocytogenes*

Examination for *L. monocytogenes* was done in accordance with ISO 11290-1:2004. From each fecal sample, 1 g was enriched (24 h, 30 °C) in 10 ml of Fraser broth with half Fraser supplement (Oxoid AG). From the first enrichment, 0.1 ml were incubated (24 h, 37 °C) in 10 ml of Fraser broth with Fraser supplement (Oxoid AG). The second enrichment was then streaked onto chromogenic *Listeria* agar supplemented with *Listeria* selective supplement and *Listeria* differential supplement (Oxoid AG) and incubated for 48 h at 37 °C. Suspicious colonies were streaked onto sheep blood agar (Difco™ Columbia Blood Agar Base EH, Becton Dickinson AG, Allschwil, CH; 5% sheep blood SB055, Oxoid AG) for appraisal of hemolysis (CAMP test with *Staphylococcus aureus* and *Rhodococcus equi*).

3.4. *Escherichia coli* harboring *stx* and *eae*

From each fecal sample, 1 g was enriched (18–24 h, 37 °C) in 10 ml of modified tryptic soy broth (Oxoid AG) with 16 mg/l novobiocin (Sigma-Aldrich Chemie GmbH, Buchs, CH). From 50 µl of the enrichment broth, a lysate was made (lysis tube, Pall GeneDisc Technologies, Bruz, F). Using the commercially available closed GeneDisc® system (Pall GeneDisc Technologies), samples were screened by real-time PCR for *stx* and *eae* (Hofer et al., 2012). To compare the occurrence of *stx*-, *eae*-, or *stx*- and *eae*-positive samples among species and age groups, contingency tables (Fisher's exact test) were used.

From *stx*-positive enrichments, subsets were streaked onto sheep blood agar and incubated for 18–24 h at 37 °C. Five randomly selected colonies (resembling *E. coli*) were subcultured on sheep blood agar (18–24 h, 37 °C), confirmed biochemically as *E. coli*, and then tested by real-time PCR (LightCycler 2.0, Roche Diagnostics AG, Rotkreuz, CH) for the presence of *stx*1, *stx*2, and *eae* (Hofer et al., 2012).

3.5. Total viable counts and *Enterobacteriaceae*

Pooled swabs from each carcass were homogenized in 40 ml of 0.85% saline solution. Suspensions (and 10^{-1} and 10^{-2} dilutions) were plated with a spiral plater (Eddy Jet, IUL SA, Barcelona, E) onto plate count agar (Oxoid AG; 48–72 h, 30 °C, aerobic

conditions) and violet red bile glucose agar (BBL, Cockeysville, MD, USA; 48 h, 30 °C, anaerobic conditions). The detection limit was 5 CFU cm^{-2} and colony counts were expressed as log CFU cm^{-2}. For counts below the detection limit, a log value of zero was used. To analyze differences (TVC, *Enterobacteriaceae* counts) between animal species and abattoirs, analysis of variance and the Bonferroni procedure were performed (Stat View 4.02, Abacus Concepts Inc., Berkeley, CA, USA). To compare *Enterobacteriaceae* occurrence among species and abattoirs, contingency tables (Chi square test, Fisher's exact test) were used.

4. Results

4.1. *Salmonella* spp. and *Listeria monocytogenes*

All 239 fecal samples collected from hunted wild red deer (n=84), roe deer (n=64), chamois (n=64), and ibex (n=27) tested negative for *Salmonella* spp. and *L. monocytogenes*. *Listeria* of other species were found in 11 (4.6%) fecal samples. These 11 samples originated from six (7.1%) red deer, four (6.3%) roe deer, and one (1.6%) chamois.

4.2. *Escherichia coli* harboring *stx* and *eae*

Of the 239 fecal samples collected from hunted wild red deer, roe deer, chamois, and ibex, 78 (32.6%) tested positive for *stx* only, 16 (6.7%) for *eae* only, and 33 (13.8%) for *stx* and *eae*. The proportion of *stx*-, *eae*-, and both *stx*- and *eae*-positive samples ranged from 23.4% (chamois) to 39.1% (roe deer), 3.7% (ibex) to 7.8% (roe deer and chamois), and 0% (ibex) to 21.4% (red deer), respectively (Table 1). Statistically significant differences between the animal species were only found amongst the samples that were both *stx*- and *eae*-positive ($P<0.05$). Within each species and category (*stx*-, *eae*-, or *stx*- and *eae*-positive), the percentage of positive samples did not statistically differ between the defined age groups (juvenile, <1 year of age; adult, >1 year of age).

Out of the 111 fecal samples in which *stx* genes were detected (alone or in combination with *eae*), 56 strains of Shiga toxin-producing *E. coli* (STEC) were isolated from 18 red deer, 19 roe deer, 13 chamois, and six ibex (Table 2). Twenty-five (44.6%) strains harbored genes for only the Shiga toxin (Stx)2 group, 17 (30.4%) for only the Stx1 group, and 12 (21.4%) for Stx1 and Stx2. With regard to the animal species, *stx*2

predominated in strains from red deer, *stx*1 or *stx*2 were found to an equal extent in strains from roe deer, and strains from chamois and ibex frequently harbored both *stx*1 and *stx*2. Two of the STEC strains (positive for *stx*1 or *stx*2) also harbored *eae* and were isolated from red deer.

4.3. Total viable counts and *Enterobacteriaceae*

To compare the carcass results from each species of wild ruminants, TVC and *Enterobacteriaceae* results were depicted as box plots, which allow analyzing median values, 50% and 80% ranges, and extreme values (Fig. 1 and 2). Mean log TVC from 136 red deer carcasses, 122 roe deer carcasses, and 70 chamois carcasses were 4.0, 4.1, and 4.2 log CFU cm^{-2}, respectively ($P>0.05$). Mean values from the different abattoirs ranged from 2.8–4.9 log CFU cm^{-2} for red deer, 3.3–4.7 log CFU cm^{-2} for roe deer, and 2.5–5.0 log CFU cm^{-2} for chamois (Table 3). TVC from abattoir D (red deer, chamois), abattoir F (roe deer), and partly abattoir C differed significantly from TVC of the other abattoirs ($P<0.05$).

Enterobacteriaceae were detected on carcasses from 119 (87.5%) red deer, 109 (89.3%) roe deer, and 63 (90%) chamois ($P>0.05$). Mean log *Enterobacteriaceae* counts were 2.3, 2.6, and 2.6 log CFU cm^{-2} for red deer, roe deer, and chamois, respectively ($P>0.05$). Mean values from the different abattoirs ranged from 0.7–3.8 log CFU cm^{-2} for red deer, 1.1–3.6 log CFU cm^{-2} for roe deer, and 0.7–3.4 log CFU cm^{-2} for chamois (Table 4). With regard to *Enterobacteriaceae* counts and prevalence, abattoir D (red deer, chamois) and abattoir F (roe deer) yielded the lowest results and differences were mainly significant ($P<0.05$).

5. Discussion

To assess the shedding of selected bacterial foodborne pathogens, fecal samples from 239 hunted wild red deer, roe deer, chamois, and ibex were examined for *Salmonella* spp., *L. monocytogenes* and *E. coli* harboring *stx* or *eae*. *Salmonella* are still a major cause of foodborne diseases (EFSA/ECDC, 2011), but comparable to our results *Salmonella* were not or only rarely detected in feces from wild ruminants (Wahlström et al., 2003; Lillehaug et al., 2005; Renter et al., 2006; Paulsen et al., 2012). Similarly, we detected no *L. monocytogenes*, albeit other *Listeria* species were occasionally found.

However, with regard to the occurrence of *Listeria* on carcasses and meat, later production stages (game chambers, cold storage rooms, processing factories) must also be considered as source of contamination (Paulsen et al., 2003; Atanassova et al., 2008).

Genes for Shiga toxins and intimin were detected in a remarkable prevalence in the hunted wild ruminants. Overall, 32.6% of the fecal samples tested positive for *stx*, 6.7% for *eae*, and 13.8% for both *stx* and *eae*. The production of one or more Shiga toxins (Stx1, Stx2, and variants) characterizes STEC. Intimin mediates attaching and effacing lesions on intestinal epithelial cells and it is present in enteropathogenic *E. coli* (EPEC) and some STEC. STEC, especially the serotype O157:H7, are responsible for a number of human diseases including diarrhea, hemorrhagic colitis, and the life-threatening hemolytic-uremic syndrome (Kaper et al., 2004; Tarr et al., 2005). Human STEC infections are frequently correlated with contaminated food and deer meat has also been implicated in the transmission of STEC to humans (Keene et al., 1997; Rabatsky-Ehr et al., 2002; Ahn et al., 2009; Rounds et al., 2012). Our results suggest that wild ruminants also constitute a STEC and EPEC reservoir, a finding supported by other studies (Asakura et al., 1998; Lehmann et al., 2006; Sánchez et al., 2009; Bardiau et al., 2010; Kistler et al., 2011). Nevertheless, direct comparison of our results with the literature is hampered by varying detection procedures used. In addition, it must be mentioned that many other surveys of wild ruminants focused only on *E. coli* O157:H7.

STEC strains pathogenic for humans tend to feature Stx2 and other virulence traits as the adhesion factor intimin (Friedrich et al., 2002; Brooks et al., 2005). Amongst our 56 STEC strains isolated from hunted wild ruminants, *stx*2 was the predominant *stx* gene identified, especially in strains from red deer. Overall, 38 strains (67.8%) were positive for *stx*2 alone or in combination with *stx*1. STEC from wild ruminants can harbor *stx*1 and *stx*2, but *stx*2 predominated in several studies (Asakura et al., 1998; Lehmann et al., 2006; Sánchez et al., 2009; Bardiau et al., 2010; Kistler et al., 2011). On the other hand, intimin genes were only detected in two (3.6%) STEC strains from hunted wild red deer in the present study. In the few other studies not focusing on *E. coli* O157:H7, *eae* was also rarely found in STEC from wild ruminants (Lehmann et al., 2006; Sánchez et al., 2009; Bardiau et al., 2010). Hence, the risk of STEC transmission from wild ruminants to humans (via consumption of undercooked venison or other fecally contaminated foods) and to domestic ruminants (via sharing of the same pastures) must not be neglected. To

assess the actual pathogenicity of the isolated STEC for humans, further strain characterization is required (Miko et al., 2009).

In addition, 328 dehided carcasses from hunted wild red deer, roe deer, and chamois were examined for TVC and *Enterobacteriaceae* by swabbing (neck, brisket, flank, rump). For the examined species of wild ruminants, mean log TVC (4.0–4.2 log CFU cm^{-2}), mean log *Enterobacteriaceae* counts (2.3–2.6 log CFU cm^{-2}), and *Enterobacteriaceae* detection rates (87.5–90%) were at comparable levels. On the other hand, the microbial status of carcasses differed considerably between certain abattoirs. Remarkable low values were present on red deer and chamois carcasses from abattoir D and on roe deer carcasses from abattoir F. Comparability of our results with published data is hampered by differences in terms of process stages used for sample collection, carcass sampling sites, and sampling methods applied. Surveys of game (mainly red and roe deer) in Austria reported ranges of several orders of magnitude in the bacterial counts (Deutz et al., 2000; Paulsen et al., 2003; Paulsen and Winkelmayer, 2004; Deutz et al., 2006): Using swabs of the body cavity or the inside of thighs, median values ranged from 4.0–6.7 log CFU cm^{-2} for TVC and 2.4–4.3 log CFU cm^{-2} for *Enterobacteriaceae*. Considerably lower counts were recently obtained in a German study investigating hunted game, including red and roe deer carcasses (Atanassova et al., 2008): Average TVC from excision samples accounted for 2.9 (red deer) and 2.6 log CFU cm^{-2} (roe deer), whereas the average *Enterobacteriaceae* value was 2.1 log CFU cm^{-2}. The results reported by Atanassova et al. (2008) were within the legal range for carcasses from domestic ruminants (Reg. [EC] No. 2073/2005), but it must be emphasized that samples were obtained from freshly shot game under well-defined conditions. Basically, when game meat is placed on the market the same way as that from farm animals, it can be reasonable to adopt the limits specified for fresh meat from domestic ruminants (Paulsen, 2011).

In the present study (and the mentioned Austrian studies), TVC and *Enterobacteriaceae* values from carcasses of hunted wild ruminants were higher than those reported for slaughtered domestic ruminants (Zweifel et al., 2005; Zweifel et al., 2008). Especially *Enterobacteriaceae*, which are used as indicators of fecal contamination (and thereby for important bacterial pathogens), were found in remarkable frequencies and counts. This might be partly due to the difficulty to warrant a hygienically correct evisceration in the field, in particular if the shooting has lacerated the

animal's intestines (Gill, 2007; Paulsen, 2011). In contrast to the (industrial) slaughter of domestic ruminants, the hunter eviscerates the shot animal before dehiding and often while it is lying on the ground. Hence, raising awareness of the hunters for their important role in the determination of the initial microbial contamination and its impact on the safety and quality of game is necessary. On the other hand, facilities dehiding, cooling and processing carcasses from wild ruminants must be aware of the associated challenges in order to avoid contaminations.

Consequently, strict compliance with good hunting and hygiene practices during any step from shooting, through evisceration in the field, to dehiding and processing is of central importance to avoid contaminations and to prevent foodborne pathogens carried by the animals (in their intestines or hides) from entering the food chain. Such measures ensure both public health protection and meat quality. The low microbial results observed on carcasses from certain abattoirs show that carcasses from hunted wild ruminants can be of a good hygienic status.

6. References

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7. Tables

Table 1

Detection of *stx* and *eae* genes in fecal samples obtained from hunted wild red deer, roe deer, chamois, and ibex (juvenile, <1 year of age; adult, >1 year of age).

Origin/ Age group	No. of samples	*stx* $^{+}$	*eae* $^{+}$	*stx* $^{+}$ and *eae* $^{+}$
Red deer	**84**	**31 (36.9%)**	**5 (6.0%)**	**18 (21.4%)**
Juvenile	22	10 (45.5%)	0 (0.0%)	5 (22.7%)
Adult	62	21 (33.9%)	5 (8.1%)	13 (21.0%)
Roe deer	**64**	**25 (39.1%)**	**5 (7.8%)**	**12 (18.8%)**
Juvenile	24	11 (45.8%)	1 (4.2%)	3 (12.5%)
Adult	40	14 (35.0%)	4 (10.0%)	9 (22.5%)
Chamois	**64**	**15 (23.4%)**	**5 (7.8%)**	**3 (4.7%)**
Juvenile	9	2 (22.2%)	2 (22.2%)	0 (0.0%)
Adult	55	13 (23.6%)	3 (5.5%)	3 (5.5%)
Ibex (adult)	**27**	**7 (25.9%)**	**1 (3.7%)**	**0 (0.0%)**
Total	**239**	**78 (32.6%)**	**16 (6.7%)**	**33 (13.8%)**

Table 2

Detection of *stx*1, *stx*2, and *eae* genes amongst 56 strains of Shiga toxin-producing *Escherichia coli* isolated from fecal samples of hunted wild red deer (n=18), roe deer (n=19), chamois (n=13), and ibex (n=6).

Origin	No. of strains	*stx*1	*stx*2	*eae*
Red deer	13	-	+	-
	2	+	-	-
	1	+	+	-
	1	+	-	+
	1	-	+	+
Roe deer	9	+	-	-
	9	-	+	-
	1	+	+	-
Chamois	7	+	+	-
	4	+	-	-
	2	-	+	-
Ibex	3	+	+	-
	2	+	-	-
	1	-	+	-

Table 3

TVC results (log CFU cm^{-2}) on carcasses obtained from hunted wild red deer, roe deer, and chamois at six small-scale abattoirs.

Abattoir (total No of sampled carcasses)	Red deer carcasses[a]			Roe deer carcasses			Chamois carcasses		
	n	$\bar{x}$	S.D.	n	$\bar{x}$	S.D.	n	$\bar{x}$	S.D.
Abattoir A (n=121)	63	3.95	0.62	33	4.52	0.69	25	4.32	0.89
Abattoir B (n=102)	44	3.88	0.84	36	4.09	0.82	22	3.90	0.53
Abattoir C (n=53)	19	4.90	0.95	18	4.72	0.70	16	4.96	0.59
Abattoir D (n=14)	8	2.81	0.68	1	3.48	nd	5	2.51	1.61
Abattoir E (n=11)	2	4.79	0.20	7	4.13	0.74	2	4.63	0.64
Abattoir F (n=27)	na[b]	nd[c]	nd	27	3.30	0.63	na	nd	nd
Total (n=328)	**136**	**4.01**	**0.87**	**122**	**4.12**	**0.87**	**70**	**4.22**	**0.99**

[a] n: No of samples; $\bar{x}$: mean log CFU cm^{-2}; S.D.: standard deviation.

[b] na: not available; [c] nd: no data for calculation.

Table 4

Enterobacteriaceae results (log CFU cm^{-2}) on carcasses obtained from hunted wild red deer, roe deer, and chamois at six small-scale abattoirs.

Abattoir (total No of sampled carcasses)	Red deer carcasses[a]			Roe deer carcasses			Chamois carcasses		
	n	$\bar{x}$	S.D.	n	$\bar{x}$	S.D.	n	$\bar{x}$	S.D.
Abattoir A (n=121)	63	2.14	1.06	33	3.20	0.91	25	2.93	0.95
Abattoir B (n=102)	44	2.12	1.36	36	2.60	1.19	22	2.06	1.14
Abattoir C (n=53)	19	3.82	1.33	18	3.63	1.11	16	3.42	0.96
Abattoir D (n=14)	8	0.73	1.02	1	3.06	nd	5	0.68	1.53
Abattoir E (n=11)	2	3.18	0.08	7	2.98	1.50	2	3.36	1.16
Abattoir F (n=27)	na[b]	nd[c]	nd	27	1.08	1.12	na	nd	nd
Total (n=328)	**136**	**2.30**	**1.38**	**122**	**2.60**	**1.40**	**70**	**2.62**	**1.28**

[a] n: No of samples; $\bar{x}$: mean log CFU cm^{-2}; S.D.: standard deviation.

[b] na: not available; [c] nd: no data for calculation.

8. Figures

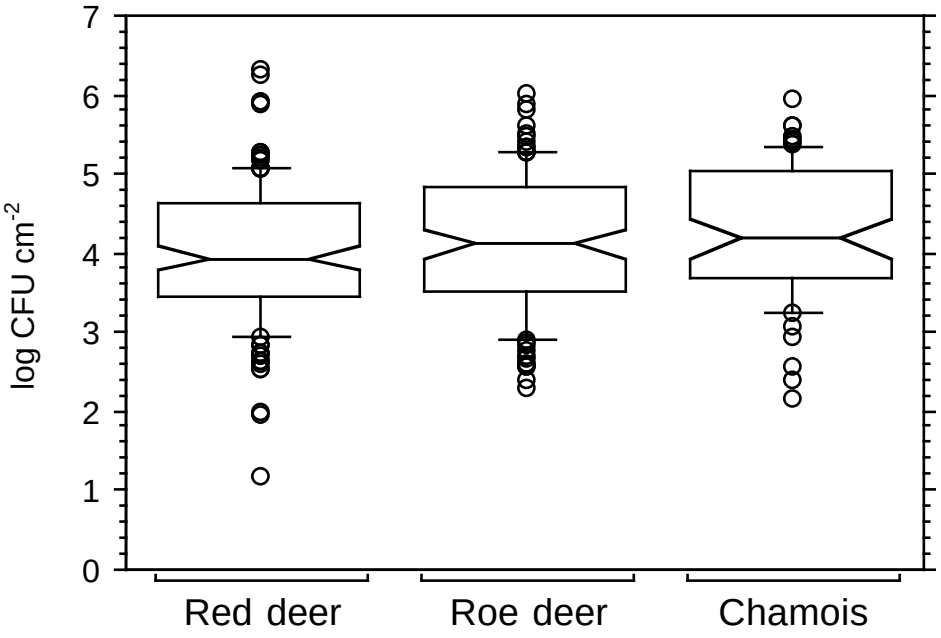

Fig. 1. Total viable counts (TVC) results (log CFU cm^{-2}) on carcasses from 136 hunted wild red deer, 122 hunted wild roe deer, and 70 hunted wild chamois.

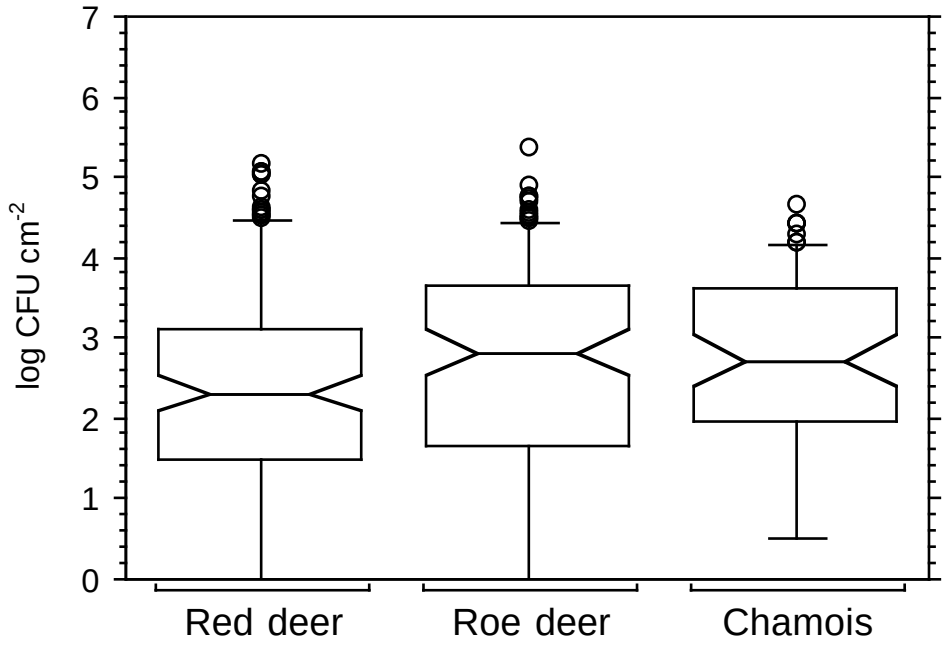

Fig. 2. *Enterobacteriaceae* results (log CFU cm^{-2}) on carcasses from 136 hunted wild red deer, 122 hunted wild roe deer, and 70 hunted wild chamois.

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